# FABRICATION OF CHITOSAN BASED NANOFIBROUS SCAFFOLD USING FREE SURFACE ELECTROSPINNING FOR TISSUE ENGINEERING APPLICATION

*A Thesis submitted in partial fulfilment of the requirements for the degree of*

***Master of Technology***

***In***

**Biotechnology**

**By**

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# National Institute of Technology, Rourkela

## CERTIFICATE

This is to certify that the thesis entitled "**Fabrication Of Chitosan Based Nanofibrous Scaffold Using Free Surface Electrospinning For Tissue Engineering Application**" by **PARINITA AGRAWAL (211BM2223)** submitted to the National Institute of Technology, Rourkela for the award of Master of Technology in Biotechnology during the session 2011-2013 is a record of bonafide research work carried out by her in the Department of Biotechnology and Medical Engineering under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

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# ABSTRACT

Tissue engineering offers a promising approach for repair of defective tissues and organs. Developing scaffold from a variety of polymer blends or composites allow adjusting the properties desired for mimicking required disinformity. In recent years, considerable attention has been given to develop chitosan based biomaterials for their applications in the field of tissue engineering due to its minimal foreign body reactions, an intrinsic antibacterial nature, biocompatibility, biodegradability, and the ability to be molded into various geometries and forms such as porous structures that are suitable for cell ingrowth and osteoconduction. The present work involves the preparation of nanofibrous mat from chitosan blended with other biopolymers such as silk fibroin, poly-vinyl alcohol and polyethylene oxide by free surface electrospinning method. The morphology and functional characterization of the developed scaffolds were performed by SEM and FTIR studies. The average fiber diameter of 269nm and 122nm were obtained with chitosan/polyvinyl alcohol and chitosan/silk fibroin, poly ethylene oxide blends respectively. Crystalline nature of the scaffolds was confirmed by XRD studies. The scaffolds are also shown to have desired biodegradable and biocompatible properties. Chitosan based polymeric scaffolds are thus proved to be potential materials for tissue engineering applications.



**Keywords**: Chitosan, Tissue engineering, free surface electrospinning, biocompatibility, biodegradability

# *Acknowledgement*

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**Parinita Agrawal**

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# Abbreviations

| | |
|---|---|
| %T | Percent Transmittance |
| %wt | weight percent (quantity) |
| µl | micro litres |
| µm | micro metre |
| 3D | three Dimentional |
| AA-water | Acetic Acid water |
| cm | centi metre |
| CS | Chitosan |
| d/w | Distilled Water |
| DMEM | Dulbecco's Modified Eagle Medium |
| FBS | Fetal Bovine Serum |
| FTIR | Fourier Transform Infra Red spectroscopy |
| g | gram |
| h | hour |
| KBr | Potassium Bromide |
| KCl | Potassium Chloride |
| kV | kilo Volt |
| L | Litre |
| M | Molar |
| Min | minute |
| ml | mili litre |
| mm | mili metre |
| MPa | Mega Pascal |
| N | Normality |
| NaCl | Sodium Chloride |
| nm | nano metre |

| | |
|---|---|
| $^{o}C$ | degree Celcius (Temperature) |
| Pa | Pascal |
| PBS | Phosphate Buffer Saline |
| PEO | Poly ethylene oxide |
| pH | hydrogen ion concentration |
| PVA | Poly Vinyl Alcohol |
| rpm | revolution per minute |
| sec | seconds |
| SEM | Scanning Electron Microscopy |
| SF | Silk Fibroin |
| v/s | versus |
| v/v | volume by volume |
| w/w | weight by weight |
| wrt | with respect to |
| XRD | X-Ray Diffraction |

**CHAPTER- 1**

.....

# *Introduction*

Bone and cartilage defects and lesions occur in variety of clinical situations. Patients are treated by mainly three ways by applying autograft, allograft or xenograft, each of these have inbuilt disadvantages. Since there is always a chance that the grafted tissue may not work as expected in the patient, allograft and xenografts suffer additional problem of donor scarcity, disease transmission or contamination and immune rejection. Tissue engineering provides a lasting cure for this by offering a biocompatible replaceable tissue having functional and mechanical integrity [1].

Tissue engineering involves scaffold designing having composition, structure, mechanical, biological and physiochemical features analogous to natural bone and mimics its extra cellular matrix (ECM) [2-4]. Like specific surface area and pore size are important for initial cell adhesion, improved cell migration provided by scaffolds with pores above 300 micron are significant in case of scaffold designed for bone or cartilage tissue growth. An added advantage of the larger pores is a reduction in cell aggregations that develop along the edges of the scaffolds. Study by Murphy et al showed that scaffolds with a mean pore size of 325 micron were optimal for bone tissue engineering [5]. By facilitating capillary formation, pores greater than ~300 μm lead to direct osteogenesis while pores smaller than ~300 μm can encourage osteochondral ossification. However, larger pores may compromise the mechanical properties of the scaffolds by increasing void volume. Scaffolds for osteochondral tissue regeneration should be non-immunogenic, non-toxic, biocompatible and biodegradable. The scaffold should possess an interconnected and spread porosity (usually exceeding 90%) with a highly porous surface and microstructure. This would allow in vitro cell adhesion, ingrowth and reorganization and would provide the necessary space for neo-vascularization in vivo. The scaffold should have sufficient mechanical strength during *in vitro* culturing to maintain the spaces required for cell ingrowth

and matrix formation. Pore size and orientation is shown to influence the mechanical properties of chitosan (CS) scaffolds. Tensile testing of hydrated samples showed that porous membranes have greatly reduced elastic moduli (0.1–0.5 MPa) compared to non-porous membranes (5–7MPa) [11]. Moreover, a scaffold must provide sufficient temporary mechanical support, matching the mechanical properties of the host tissue as closely as possible, to bear in vivo stresses and loading. It is possible to realize scaffolds with tailored physical, biological and mechanical properties by combining bioabsorbable polymers and bioactive ceramic phases**.**

Numerous natural and synthetic polymers have been investigated, Chitosan has attracted attention of many researchers because of its biodegradable, biocompatible, and non-toxic properties and thus proposed as a safer material for use in biomedical applications [6-9]. A.Di. Martino *et al* found that CS possesses intrinsic antibacterial activity [11]. Studies have shown that CS can reduce the infection rate of experimentally induced osteomyelitis by Staphylococcus aureus in rabbits. Its cationic amino group associates with anions on the bacterial cell wall, suppressing biosynthesis and disrupts the mass transport across the cell wall accelerating bacterial death. Due to this antibacterial property it has been blended with other polymers in various biomedical related studies. CS has also been reported to combine with a variety of delivery materials such as alginate, hydroxyapatite, hyaluronic acid, calcium phosphate, PMMA, poly-L-lactic acid (PLLA), and growth factors for potential application in orthopedic tissue engineering.

In recent years, polymer blending has become a method for providing polymeric materials with desirable properties for practical applications. In particular, chitosan blended with PVA has been reported to have good mechanical and chemical properties and, as a topic of great interest, has been studied in the biomedical field [10]. The enhanced property has been attributed

to the interactions between chitosan and PVA in the blend through hydrophobic side-chain aggregation and intermolecular and intra-molecular hydrogen bonds.

The other important factor is the fabrication method. While the fabrication of porous scaffold has been the choice of many researchers, the fabrication of scaffold from nanofibres generated by electrospinning is gaining importance in recent years. **Electrospinning** is a simple and easy way to control the morphology of ultrafine fibers. In this high voltage electric field is used. The fibers produced by this method have characteristics, such as very large surface-to-volume ratio and a high porosity with a small pore size [23,24], pore distribution is irregular in the matrix. Therefore, there is need of systematic research effort to prepare electrospun nanofibres from polymeric blends of chitosan with other biopolymers.

**Scaffolds in Tissue Engineering:**

One of the most attractive domains of tissue engineering is development of scaffold, a three dimentional porous solid structure which has a key role in supporting tissue regeneration [1]. The ultimate goal of the scaffold design is the production of an ideal structure that can replace the natural ECM until host cells can repopulate and resynthesize a new natural matrix. To achieve this goal, the scaffold material must be selected carefully, and the scaffold architecture must be designed to insure that the seeded cells are biocompatible with the engineered scaffold [2].

Purpose of a scaffold in tissue engineering is to provide analogous structural framework as well as imitate the micro-environment of the tissue meant to be repaired / regenerated. Ideally, a scaffold should possess properties like biocompatible, bioactive, biodegradable, porous and possess adequate mechanical strength as per the target biological site. Bioactivity of a scaffold helps in cell-biomaterial interactions, cell proliferation, adhesion growth, migration and differentiation along with extracellular matrix deposition and nutrient and gases transfer and waste removal, ultimately leading to cell survival. Biodegradability contributes to the

replacement of biological tissues by toxic waste products free physiological extracellular components. The main requirement in this case is the rate of degradation of the scaffold should match the rate of new tissue formation to maintain the structural integrity of the tissue, and smooth and successful load transfer from cells to the tissue [3]. Porosity is required for accommodating cell differentiation and proliferation, which eventually leads to functional tissue formation [3,4]. Pores should also be interconnected to facilitate uniform cell seeding and their successful distribution, this also helps in successful nutrient and metabolites exchange in the cell-scaffold contructs [5-7]. A scaffold should possess adequate mechanical stability to withstand mechanical forces experienced by the cell-scaffold construct during implantation procedure and post-implantation during person's normal activities or by any kind of internal forces experienced by body fluids. A major concern with biodegradable polymers is they loose their mass and mechanical integrity during degradation, this leads to their poor mechanical properties. For hard tissue applications like bone, a scaffold should possess adequate Young's modulus and strength [8,9].

**Polymers used in Tissue Engineering:**

Numerous natural and synthetic polymers have been investigated. **Natural polymers** are classified as proteins (silk, collagen, gelatin, fibrinogen, elastin, keratin, actin and myosin), polysaccharides (cellulose, amylose, dextran, chitin and glycosaminoglycans) and polynucleotides (DNA, RNA). The macromolecular similarities of natural polymers with natural tissues generally increase biocompatibility and reduce immunologic responses. **Synthetic polymers** have found increased applications as they possess appreciable mechanical and physical properties such as tensile strength, elastic modulus and degradation rate. Typical biodegradable polymers used for biomedical purposes are hydrophobic polyester, such as polyglycolide (PGA) and polylactide (PLA), polyurethanes (PUs) and polyamides (PAs). Synthetic biodegradable polmers like PVA (poly vinyl alcohol), PGA (poly glycolic acid), PLA (poly lactic acid) and their copolymers are used in various clinical applications.

Biodegradable polymers are widely accepted as suitable materials because of their biocompatibility and ease of processability [10,11]. Biodegradable polymers degrade through the process of hydrolysis and are absorbed by human body, whose place is then taken by the ECM or supportive tissue. **Composite materials** can be defined as a material composed of two or more

chemically and physically distinct phases (metallic, ceramic or polymeric), which are separated by an interface. Researchers have been applying composite materials in tissue engineering to enhance mechanical properties and cell function, and deliver special molecules. Composites aim to combine the properties of both materials to enhance tissue reconstruction. Chitosan has attracted attention of investigators because of its biodegradable, biocompatible, and non-toxic properties; thus, it has been proposed as a safer material for use in biomedical applications [12,13]. Chitosan nanofibres are being generated by employing chitosan alone or in combination with other biomaterials by electrospinning.

In this study, CS was blended with PVA to form a scaffold. Polyvinyl alcohol has excellent film forming, emulsifying and adhesive properties. It is also resistant to oil, grease and solvents. It is odorless and nontoxic. It has high tensile strength and flexibility, as well as high oxygen and aroma barrier properties. However these properties are dependent on humidity, in other words, with higher humidity more water is absorbed. The water, which acts as a plasticiser, will then reduce its tensile strength, but increase its elongation and tear strength. PVA is fully degradable and dissolves quickly. PVA has a melting point of 230°C and 180–190°C (356-374 degrees Fahrenheit) for the fully hydrolysed and partially hydrolysed grades, respectively. It decomposes rapidly above 200°C as it can undergo pyrolysis at high temperatures.

Silk is a typical fibrous protein produced by a variety of insects including silkworm. Silk consists of two types of proteins, fibroin and sericin. Fibroin is the protein that forms the filaments of silkworm silk and can be regenerated in various forms, such as gels, powders, fibers, or membranes, depending on application. Silk fibroin (SF) is one of the candidate materials for biomedical applications, because it has several distinctive biological properties including good biocompatibility, good oxygen and water vapour permeability, biodegradability, slow degradation profile and minimal inflammatory reaction [14]. The amino acid sequence of SF contains repetitive glycine-alanine-glycine-alanine-glycine-serine (GAGAGS) sequence, which self-assembles into anti-parallel β-sheet structure [15]. These β-sheets are highly crystalline and crosslink the polymer through strong inter and intra-molecular hydrogen bonds, providing the material good mechanical strength. However, tensile properties of SF could be improved by blending with other polymers, such as Poly ethylene oxide (PEO). PEO is widely used in biomedical applications [16,17], due to its non-toxicity, easy dissolution in organic and aqueous

solvents and easy excretion by hepatic and renal pathways [18]. Particularly, PEO can be used as a processing aid, easing electrospinning of materials for which this procedure is normally not allowed [19,20]. It also plays a role in improving fiber properties and functionalities [21,22].

Chitosan is an attractive biopolymer that has excellent wound healing, property thus making it suitable for tissue engineering. However, so far there is only limited literature available on the incorporation of Chitosan into the Silk/PEO nanofibrous structure, its effects on the physiochemical structure and the properties of SF-CH particularly the *in vivo* biological responses to tissue forming. Therefore a systematic investigation to assess the structural, mechanical and biological effects of the incorporation of Chitosan into PVA and then into Silk/PEO matrix was be carried out in the present study.

**Processes in Tissue Engineering:**

Different types of scaffolds can be prepared depending upon different processes followed for their fabrication. Mainly two types of scaffolds can be formed, porous and fibrous. Different processes applied in tissue engineering for scaffold fabrication are Salt leaching, Solvent casting, freeze drying, Electrospinning and 3D Printing.

**Salt leaching, Solvent casting** and **freeze drying** are the methods to produce porous scaffolds. The pore distribution is largely irregular in the scaffold and the scaffolds produced possess moderate mechanical strength.

**3D printing** is a process of making a three-dimensional solid object of virtually any shape from a digital model. 3D printing is achieved using an additive process, where successive layers of material are laid down in different shapes. Several different 3D printing processes have been invented which differ in the way layers are deposited to create parts and in the materials that can be used. Some methods melt or soften material to produce the layers, e.g. selective laser sintering (SLS) and fused deposition modeling (FDM), while others cure liquid materials using different sophisticated technologies, e.g. stereolithography (SLA). The scaffolds formed by these techniques have regular pore distribution and wel defined microstructure. They possess moderate to high mechanical strength.

In this work, attempt has been given to develop nanofibres from a polymeric blend of CS with other polymers by free surface electrospinning method. The advantage of this method from needle based method is, to overcome the low productivity of nozzle-based electrospinning. In **free surface electrospinning** (also referred to as "needleless electrospinning") electrohydrodynamic jets self-organize spontaneously on a free liquid surface. This process could be modified to generate aligned nanofibres which would yield improved properties of the scaffold to be used for various tissue engineering applications.

**Bone and Cartilage Tissue Engineering:**

Cartilage tissue engineering involves scaffold designing having composition, structure, mechanical, biological and physiochemical features analogous to natural cartilage and mimics its extra cellular matrix (ECM) [26-28]. Like specific surface area and pore size are important for initial cell adhesion, improved cell migration provided by scaffolds with pores above 300 microm are significant in case of scaffold designed for bone or cartilage tissue growth. An added advantage of the larger pores is a reduction in cell aggregations that develop along the edges of the scaffolds. Study by Murphy et al showed that scaffolds with a mean pore size of 325 microm were optimal for bone tissue engineering. By facilitating capillary formation, pores greater than ~300 μm lead to direct osteogenesis while pores smaller than ~300 μm can encourage osteochondral ossification. However, larger pores may compromise the mechanical properties of the scaffolds by increasing void volume [36].

# Objectives

1. To prepare chitosan based polymer blends of desired properties to develop tissue engineered scaffold
2. To fabricate chitosan based electrospun nanofibrous mat
3. To optimize key parameters of electrospinning process
4. To characterize the nanofibrous scaffold
5. To perform *invitro* study of cell scaffold for biocompatibility and biodegradability

**CHAPTER- 2**

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# *Literature Review*

Chitosan (CS) is a copolymer of N-acetyl-d-glucosamine (Glc-NAc) and d-glucosamine (GlcN) that is produced by alkaline deacetylation of chitin. CS is a weak base, because the pKa value of the d-glucosamine residue is approximately 6.2–7.0. Due to this basicity, CS is insoluble in neutral and alkaline pH values but is soluble in acidic media. CS is biodegradable, biocompatible and non-toxic; therefore, it has been proposed as a safer and promising material for biomedical applications [12,13].

Natthan *et al* have reported that CS nanofibers are successfully generated from electrospinning of homogeneous CS or CS derivatives, like carboxymethyl CS, carboxyethyl CS, quaternized CS and hexanoyl CS [29]. However, some organic solvents or organic acids, such as 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), chloroform, trifluoroacetic acid (TFA), acrylic acid and acetic acid, could be employed in the fabrication of these homogeneous chitosan nanofibers or derivatives. Traces of these toxic organic solvents or acids in electrospun products are harmful when applied to wounded human skin or tissue. They also showed that Chitosan-based nanofibers could be successfully generated by the electrospinning of chitosan solutions blended with poly (ethylene oxide), PVA, collagen, silk fibroin, poly (l-lacticacid), poly (caprolactone) and agarose.

Scaffolds for osteochondral tissue regeneration should be non-immunogenic, non-toxic, biocompatible & biodegradable. The scaffold should possess an interconnected and spread porosity (usually exceeding 90%) with a highly porous surface and microstructure. This would allow in vitro cell adhesion, ingrowth and reorganization and would provide the necessary space for neo-vascularization in vivo. The scaffold should have sufficient mechanical strength during *in vitro* culturing to maintain the spaces required for cell ingrowth and matrix formation. Pore size and orientation is shown to influence the mechanical properties of CS scaffolds. Tensile testing of hydrated samples showed that porous membranes have greatly reduced elastic moduli (0.1–0.5 MPa) compared to non-porous membranes (5–7MPa) [30].

Moreover, it must provide sufficient temporary mechanical support, matching the mechanical properties of the host tissue as closely as possible, to bear in vivo stresses and loading. It is possible to realize scaffolds with tailored physical, biological and mechanical properties by combining bioabsorbable polymers and bioactive ceramic phases**.**

Various researches have shown that electrospun CS nanofiber mats have been successfully prepared without organic solvent or organic acids by blending CS with PVA. The weight ratio in this blend affects the viscosity and conductivity of the solution. The morphology of fibers and their diameters were strongly influenced by the composition of the solution [29].

A.Di. Martino et al found out that CS possesses intrinsic antibacterial activity. Studies have shown that CS can reduce the infection rate of experimentally induced osteomyelitis by Staphylococcus aureus in rabbits [30]. Its cationic amino group associates with anions on the bacterial cell wall, suppressing biosynthesis and disrupts the mass transport across the cell wall accelerating bacterial death. Due to this antibacterial property it has been blended with other polymers in various biomedical related studies. CS has been combined with a variety of delivery materials such as alginate, hydroxyapatite, hyaluronic acid, calcium phosphate, PMMA, poly-L-lactic acid (PLLA), and growth factors for potential application in orthopedics.

In recent years, polymer blending has become a method for providing polymeric materials with desirable properties for practical applications. In particular, chitosan blended with PVA has been reported to have good mechanical and chemical properties and, as a topic of great interest, has been extensively studied in the biomedical field. The enhanced property has been attributed to the interactions between chitosan and PVA in the blend through hydrophobic side-chain aggregation and intermolecular and intra-molecular hydrogen bonds [31, 32].

Ki et al studied the development of silk fibroin 3D scaffolds by electrospinning method. A rolling metal drum was used as a collector for sheet-like nanofibrous scaffold and a metal bath filled with methanol was used as a collector for 3-D nanofibrous scaffold. The electrospinning process was performed at room temperature and 60% humidity. Electric potential and distance to collector were fixed at 12 kV and 10 cm, respectively. The porosity of 3-D nanofibrous scaffolds was much higher than that of 2-D nanofibrous scaffolds. MTT assay confirms that 3-D nanofibrous scaffolds provides a more favorable environment for the proliferation and cellular metabolic activity of seeded osteoblasts than nanofibrous scaffolds [33].

Cai et al studied the fabrication of silk fibroin/chitosan composite nanofibers. Silk fibroin was dissolved in HFIP and Chitosan was dissolved in the mix-solvent HFIP / TFA. The silk fibroin–chitosan composite fibers with a diameter ranging from 185.5 ± 114.7 nm to 484.6 ±

410.8 nm were fabricated using electrospinning. Fiber diameters decreased with the increasing of chitosan content. The other parameters involving voltage, collecting distance, feed rate and solution concentration were fixed during electrospinning, therefore, fiber diameters are mainly dependent on the ratio of chitosan to silk fibroin. The tensile strength of the cross-linked nanofibrous membranes increased from 1.3 MPa to 10.3 MPa with the increased content of silk fibroin. The addition of silk fibroin enhanced the mechanical properties of composite nanofibrous membranes. From the MTT assay, it was found that CS/SF composite nanofibrous membranes promoted cell attachment and proliferation. The antibacterial activity increased greatly with an increasing proportion of chitosan [34].

Li et al analyzed the preparation of silk fibroin/PVA composite nanofibers.PVA has good mechanical properties and potential biomedical applications, so the addition of PVA to SF could improve mechanical properties of SF and the SF added PVA should also have potential to be biomaterials. The PVA solution and the SF/formic acid solution were mixed on the basis of mass ratios of PVA to SF which was varied from 90/10 to 70/30. The spinning solution was electrospun at 20 KV, with tip-to-collector distance 18 cm. The heat treated composite nanofibers successfully inhibit the growth of the bacteria and had strong antimicrobial activity [35].

**CHAPTER- 3**

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# *Materials and Method*

## CHEMICALS:

1) Chitosan (deacetylation degree >90%) HIMEDIA, India (RM9358)
2) Poly vinyl alcohol (PVA) (molecular weight 14000) Otto Chemika, India
3) Poly Ethylene Oxide (PEO) (molecular weight 600000) Sigma, India (182028-56)
4) Acetic acid (M 60.05g/mol) Merck, India
5) Silk Fibroin – Powder form extracted from *Bombyx mori* silk cocoons
6) LiBr
7) $Na_2CO_3$

## COMPOSITION:

| **1) SIMULATED BODY FLUID (SBF)-** | **(g/L)** |
|---|---|
| NaCl | 6.547 |
| $NaHCO_3$ | 2.268 |
| KCl | 0.373 |
| $Na_2HPO_4 \cdot 2\ H_2O$ | 0.178 |
| $MgCl_2.\ 6H_2O$ | 0.305 |
| 1M HCl | 15 mL |
| $CaCl_2.\ 2H_2O$ | 0.368 |
| $Na_2SO_4$ | 0.071 |
| $(CH_2OH_3)\ CNH_2$ | 6.057 |
| pH | 7.4 |

| 2) **PHOSPHATE BUFFER SALINE (PBS)-** | (g/L) |
|---|---|
| NaCl | 8.01 |
| KCl | 0.20 |
| $Na_2HPO_4 \cdot 2\ H_2O$ | 1.78 |
| $KH_2PO_4$ | 0.27 |
| pH | 7.4 |

## INSTRUMENTS:

| | |
|---|---|
| 1) Dialysis Cassette | Thermo Scientific, slide-A-Lyzer 10K |
| 2) Lyophilizer | |
| 3) Electrospinning machine | Elmarco, Nanospider "NS Lab 200" |
| 4) Viscometer | Bohlin Visco 88, Malvern Instruments, U.K |
| 5) Scanning Electron Microscope | JEOL-JSM 6480 LV SEM |
| 6) XRD-PANalytical | Philips Analytical |
| 7) KBr press | Technosearch |
| 8) FTIR | IR-Prestige-21 |
| 9) Vernier Calpier | Absolute Digimatic, Mitutoyo |
| 10) Universal Mechanical Tester (UTM) | Instron Electropuls E1000 |
| 11) Phase contrast Microscope | Carlzens, Alex 480 |
| 12) Weighing balance | Shimazdu |
| 13) Magnetic Stirrer | Tarsons, MC02 |

## METHOD

**Work Progress Flow Chart-**

- Chitosan dissolved in Acetic-acid-water at 2 wt% concentration.
- PVA-DW solution (10 wt%)
- Silk Fibroin-DW solution (1 wt%)

↓

**Blends Preparation**

- **Chitosan/PVA** – 10:90, 20:80, 30:70, 35:65, 40:60 and 50:50
- **Chitosan/SF -** 75:25, 50:50, 25:75 and 10:90
- **Chitosan/SF/PEO -** 1:1:1, 2:1:1 and 2:2:1 (weight ratios)

↓

**Characterization of polymer blend:**

- Rheology Analysis

↓

**Preparation of Nanofibrous scaffold by Electrospinning**
Working Distance- 11.5cm
Voltage applied- 70kV

↓

**Characterization of polymer solution and prepared scaffold:**

- Microstructure Analysis- SEM, XRD, FTIR
- Swelling Ratio and Water Uptake Capacity
- Mechanical Property testing- Tensile strength
- Biodegradation Study

↓

***In-vitro* biocompatibility study**

## Experimental Procedure:

## 1. Preparation of polymer blend:

### A. Preparation of Chitosan-PVA solutions and blends:

PVA was dissolved in distilled water (DW) at a concentration of 10 wt% and chitosan was dissolved in acetic acid-water (AA-water) solution (2 wt%) at a concentration of 2 wt%. These solutions were mixed at different weight ratios of (PVA/chitosan) 90/10, 80/20, 70/30, 65/35, 60/40 and 50/50 (5ml each).

### B. Preparation of Chitosan-Silk Fibroin Blends:

#### B.1. Preparation of SF by degumming method:

Silk Fibroin (SF) was obtained from *Bombyx mori* silkworm cocoons by Degumming method, which includes cutting the cocoons into small pieces cleaning the cocoons well and removing completely the traces of the silkworm and any other debris. The cocoons were then washed with distilled water and then boiled in 0.01 M sodium carbonate for 60 min, then washed under running distilled water thrice, to remove sericin. After overnight oven drying at $45^{o}$C, the resultant fibers were dissolved in 9.3 M Lithium bromide (LiBr) and heated at 50°C. LiBr residue was removed by dialysis, using dialysis cassette (Thermo Scientific, slide-A-Lyzer 10K) against distilled water for 3 days with water change every 3h. The dialyzed solution was freeze dried in a lyophilizer to obtain silk in dried powder form (now onwards referred as regenerated SF). The regenerated SF powder was kept in air tight container until used.

#### B.2. Silk Fibroin solution preparation and blending with CS:

Regenerated silk fibroin powder was dissolved in aqueous solution to form 1 wt% polymer solution. The solution was mixed and allowed to stir for 24 hours. CS/SF blend solutions were prepared by mixing CS and SF solutions in different ratios by volume (75:25, 50:50, 25:75 and 10:90) making final volume to 5ml. These solutions were kept on magnetic stirrer overnight after which they were electrospun.

**C. Preparation of Chitosan-SF-PEO blends:**

Poly ethylene oxide (PEO) powder was added to the CS/SF blend solutions in order to modify them and enhance the fiber formation efficiency during electrospinning. Thus CS, regenerated SF and PEO powder were mixed in weight ratios 1:1:1, 2:1:1 and 2:2:1 (CS:SF:PEO) and stirred overnight and then subjected to electrospinning.

## 2. Study of Rheological behavior of polymer blends:

Prior to electrospinning the viscosity of solutions were tested by Bohlin Visco 88 viscometer, manufactured by Malvern Instruments, U.K. In order to calculate viscosity Moore Model was applied.

## 3. Preparation of Nanofiber by Electrospnning :

Nanofibers were made by subjecting polymers to high voltage in electrospinning machine (Elmarco, Nanospider "NS Lab 200"). The samples were tested for fiber formation by keeping a drop of the sample on the sample space under varied process conditions like changing electrode to collector distance (working distance), voltage applied and electrode rotation speed. Those blend ratios which were able to form fibres were then electrospun in higher volumes for obtaining nanofibre sheets. The fibers were collected on the fabric and separated after drying which was then stored for characterization.

## 4. Study of key Electrospinning Parameters

### 4.1. Ratio of polymers in the blend.

Polyethylene oxide is added in the blend solution in order to make the CS:SF formulation electrospinnable. PEO is a synthetic polymer thus its degradation and removal from the body is an issue when used in larger amount. Lowering the amount of PEO will serve the purpose of decreased immune reaction when the cell-scaffold construct is incorporated inside the body.

Weight ratios of CS:SF:PEO were prepared keeping minimum possible ratio of PEO. CS:SF:PEO (1:1:0.4, 1:1:0.3, 1:1:0.2 and 1:1:0.1) solutions were prepared and kept for stirring overnight. Then these were electrospun to check nanofiber formation.

4.2. **Effect of Process Parameters:**

For testing optimum process parameters the electrospinning was performed under varied processing conditions namely voltage applied, (working distance) and speed of electrode rotation (rpm).

Figure 1. Nanofiber formation by free surface electrospinning

## 5. Characterization of Nanofibrous scaffold:

5.1.*Morphology analysis*:

The morphology and microstructure of the synthesized samples were evaluated using **SEM (**Scanning Electron Microscopy**)**. The electrospun fiber samples were coated with a thin layer of platinum (Pt) and their morphologies were observed under a scanning electron microscope (JEOL-JSM 6480 LV SEM) that operated at the acceleration voltage of 15 kV. Images were taken at 5000X, 10000X and 20000X magnifications.

*5.2. XRD Analysis:*

The electrospun fibers were subjected to X-rays to obtain **X-ray diffraction (XRD)** pattern in order to reveal detailed information about the chemical composition and crystallographic structure of manufactured nanofibres. The instrument used for scanning was XRD-PANalytical and range was $10^{o}$-$50^{o}$ keeping the step size 2 theta.

*5.3.FTIR Analysis:*

Molecular structure of a nanofiber can be characterized by Fourier Tranform Infra Red (FTIR). The FT-IR analysis was based on the identification of absorption bands concerned with the vibrations of functional groups presented in macromolecules [37].

The Nanofibrous polymer scaffold could not be ground into fine powder so its pellet was made. A thin fibre sheet is pressed in between the two KBr powder layers in the KBr press Technosearch instrument. This preparation is pressed till 0-10 tons and then released, this forms a pellet. This pellet is then placed in IR-Prestige-21 to record the FTIR readings, and a plot of wavenumber ($cm^{-1}$) versus percent transmittance (%T) is prepared.

*5.4.Swelling Ratio and Water Uptake Capacity:*

The equilibrium swelling ratio (Es) was measured by the conventional gravimetric method. The dry weight (Wd) of scaffold was measured and they were immersed in distilled water and incubated for 24h at $37^{o}$C. The wet weight (Ws) of the scaffold was determined by weighing it when excess water was blotted out with absorbent paper. The equilibrium swelling ratio of the scaffolds was defined as the ratio of weight increase (Ws-Wd) with respect to the initial weight (Wd) of dry samples. Each value was averaged from three parallel measurements. Es was calculated using the following equation:

$$Es= (Ws-Wd)/Wd$$

And water uptake percentage (Wu) was measured using the equation:

$$Wu= (Ws-Wd)/ Ws \times 100$$

*5.5.Mechanical Strength testing:*

Sample preparation for tensile testing- Fibre sheets were cut into specific geometry 20mm X 10mm, and a cardboard sheet was pasted at each end along the length to provide support and grip with the clamp, as shown in the figure 2. The thickness of the sheets was measured using Digital Vernier Calpier (Absolute Digimatic, Mitutoyo).

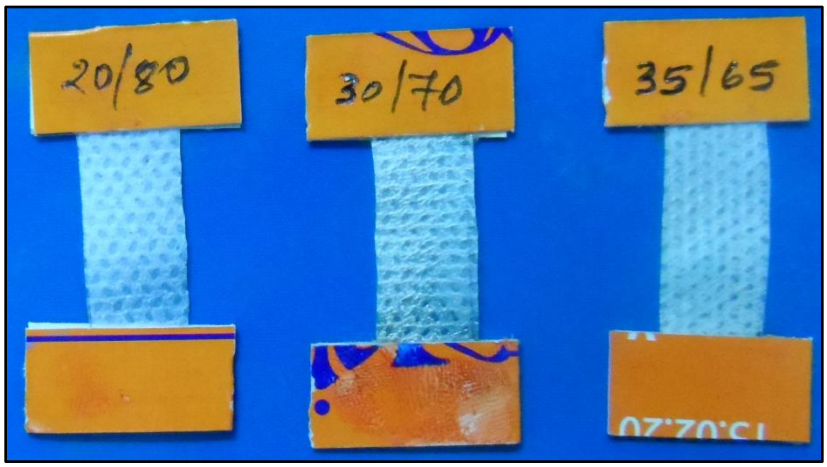


Figure 2. Sample preparation for tensile testing

Tensile Test- Tensile strength of electrospun nanofiber sheets was measured using Universal Mechanical Tester. The fiber sample was stretched with a computer controlled Instron Electropuls E1000 to test its tensile strength. After a particular load and elongation the sample breaks, and the program generates the result in the form of graph Load v/s Extension. Depending upon the feeded information regarding dimensions of the sample and the generated raw data by the program, various parameters are also shown in the result like load at break, Modulus and tensile strength of the sample. To ensure reliable result the process was performed twice for each sample. Tensile testing steps showing extension and break of the sample due to increasing load is shown in figure 3.

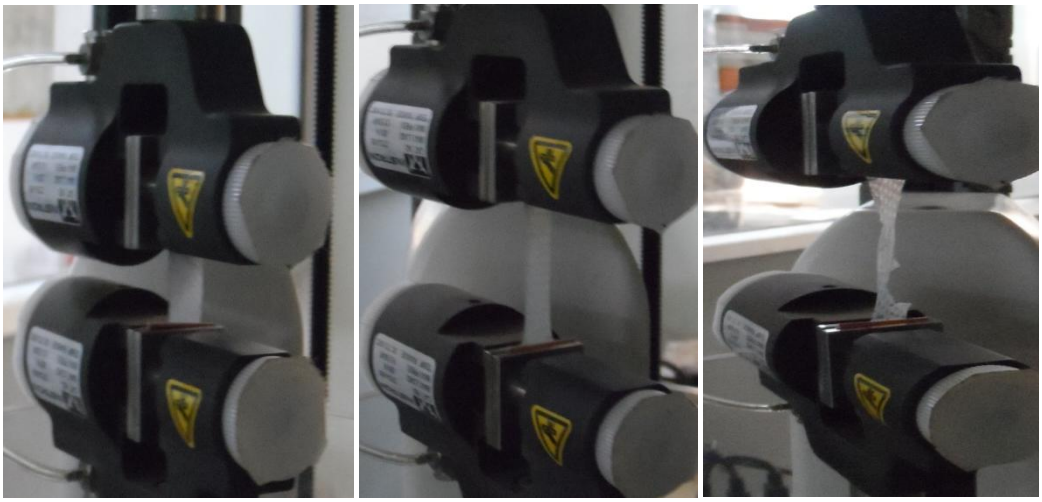

Figure 3. Tensile testing steps showing extension and break of the sample due to increasing load.

*5.6.Biodegradation Study:*

The scaffolds of known dry weights were sterilized by immersing in 70% ethanol and then in stimulated body fluid (SBF) pH 7.4 at 37°C. The SBF solution was refreshed daily to ensure continuous degradation. Samples were removed from the medium, rinsed with distilled water and weighed in every 15mins for first hour and then every 2h for 24h and then twice regularly for 1 month. The experiment was done in triplicates for each scaffold. The extent of degradation was expressed as a percentage of weight remained of the dried sample after degradation. The percentage of weight loss was calculated using the following equation:

$$\text{Weight loss} = (W_i - W_f)/W_i \times 100$$

Where, $W_i$ and $W_f$ represents the initial and final weight of scaffolds, respectively.

## 6. *In-vitro* biocompatibility study:

For studying biocompatibility of electrospun nanofibres, the cells were seeded on the scaffold. Following steps were performed for this-

i. Scaffold Sterilization- Electrospun nanofibres were sterilized by immersing in 70% ethanol for 1 h.

ii. Scaffold Neutralization- Scaffolds were neutralized by washing with PBS 3-4 times at regular intervals. pH of the solution was also checked every time PBS was changed, when the pH reached close to 7, the scaffold was considered to be neutralized.

iii. Cell preparation- Mesenchymal stem cells were trypsinized and suspended in DMEM and 10% FBS having broad spectrum antibiotic centrifuged to obtain individual cells in a suspension.

iv. Cell seeding- Cells were seeded on the sterilized nanofibers and kept for incubation at $37^{o}$C for 72hr.

**CHAPTER- 4**

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# *Results and Discussion*

1. Preparation of polymer blend:

Polymer solutions were mixed in different w/w and v/v ratios and stirred overnight on magnetic stirrer, to form clear blends, which were then characterized and processed to form nanofibres.

2. Study of Rheological behavior of polymer blends

The viscosity of pure Chitosan was 0.317 Pa sec which increased several folds after blended with PVA solution at various ratios, as shown in figure 4. This is in agreement with the data published by Paipitak *et al* who reported a linear increase in viscosity of CS solution blending with increasing amounts of PVA [38]. Blending CS with SF and PEO also showed increment in viscosity, as shown in figure 4.

Experiments performed by Alhosseini *et al* has established that the high viscosity increases the interaction of two polymers, mainly through hydrogen bonding, and decreases the effects of surface tension [31]. This will result in formation of fibers with uniform morphology after electrospinning.

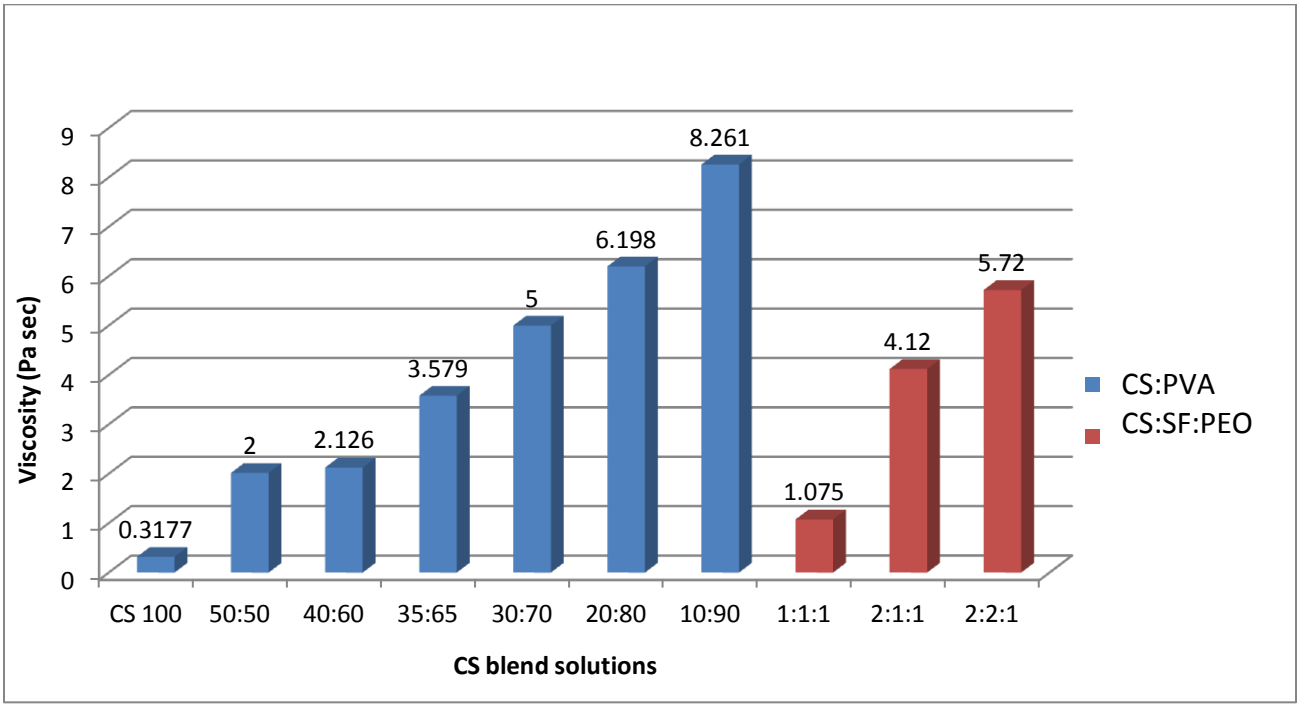



**Figure 4. Viscosity measurement of CS blend solutions**

Study of key Electrospinning Parameters:

### 4.1 Ratio of polymers in the blend.

CS:SF:PEO blend solutions were prepared keeping the minimum possible ratio of PEO and electrospun to check nanofiber formation. No fiber was formed for the blends containing less than 0.5 ratio of PEO. Result is shown in following table

Table 1. Effect of composition of polymer blend on electrospinning

| Serial no. | CS:SF:PEO blend | Nanofiber formation |
|---|---|---|
| **1.** | 1:1:0.1 | No |
| **2.** | 1:1:0.2 | No |
| **3.** | 1:1:0.3 | No |
| **4.** | 1:1:0.4 | No |
| **5.** | 1:1:0.5 | Yes |
| **6.** | 1:1:1 | Yes |

### 4.2 Effects of process parameters of electrospinning:

The parameters studied for optimization are listed in table 2.

Table 2. Effect of Applied voltage, Working Distance and electrode rotation on electrospinning

| Sample | Voltage applied (kV) | Working Disance (cm) | Electrode rotation speed (rpm) |
|---|---|---|---|
| **CS:PVA** | 70 | 11.5 | 6.8 |
| **CS:SF** | 15 to 50 | 11.5 | 7.0 |
| **CS:SF:PEO** | 60 | 12.0 | 7.0 |

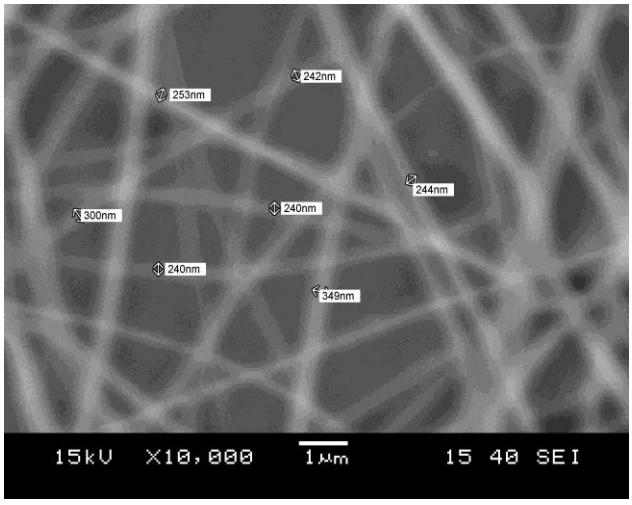
## Characterization of Nanofibrous scaffold

### *5.1.Morphology Analysis:*

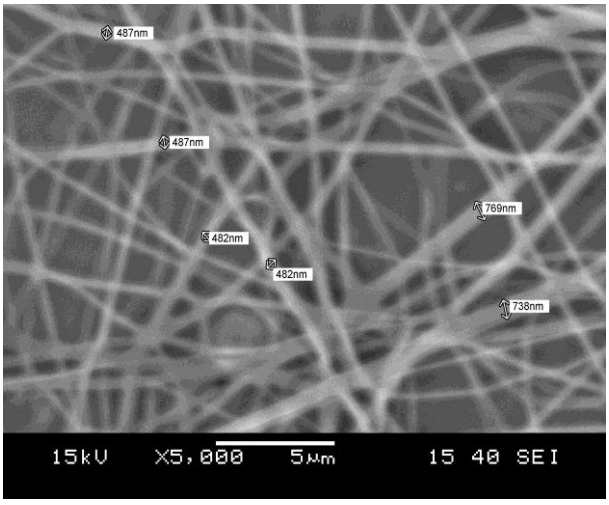
Scanning Electron Microscopy (SEM):

A. CS/PVA nanofibres- Figures 5a to 5j show the SEM micrographs of the electrospun CS/PVA nanofibers. An average fiber diameter of CS/PVA blend, weight ratio 90/10 was found to be 300 nm with a range of 240–349 nm. For blend ratios 70/30, 65/35 and 60/40, the average fiber diameter obtained was 282 nm, 264 nm and 260 nm respectively. The trend of decrease in fiber diameter with decreasing PVA concentration in the blends was observed, and with the blend ratio 50/50 (CS/PVA) resulted in fibers with beads morphology (Fig. 5i and 5j).
B. CS/SF/PEO Nanofibres- Morphology analysis of CS/SF/PEO blended scaffolds showed unaligned nanofibres formation as shown in figure 6a to 6d. Micrograph of ratio 1:1:1 scaffold at 20,000X magnification shows that the diameter of the fibers was less varied and was in the range of 122nm to 130nm. For blend ratio 2:2:1, the fiber diameter was found to be in the range of 120nm to 126nm.

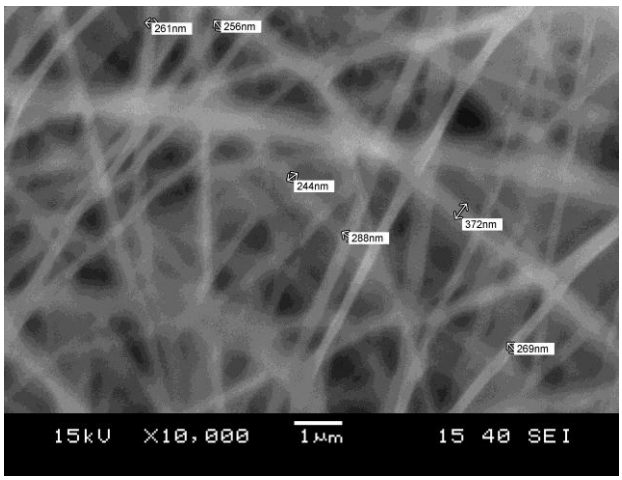
In electrospinning, the fiber diameter is dependent on the viscosity and charge of the solution. It was observed that, fiber diameter increased with increase in viscosity. CS affects not only the viscosity but also the charge density at the surface of the ejected jet through its cationic polyelectrolytic property. It increases the charge density at the surface of the jet which in turn increases the elongation force and decreases the diameter of the fiber [39].




5(a)




5 (b)

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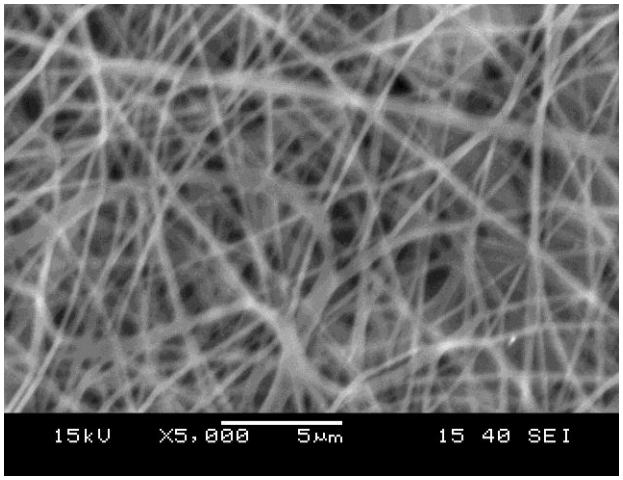


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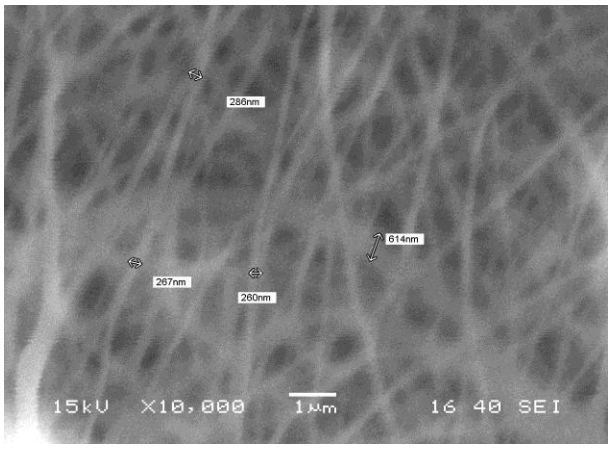


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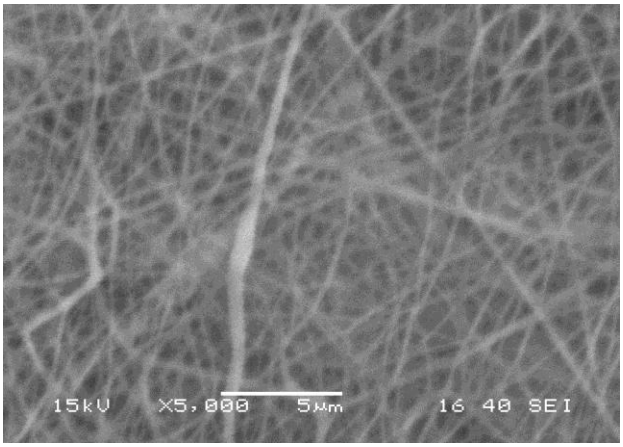


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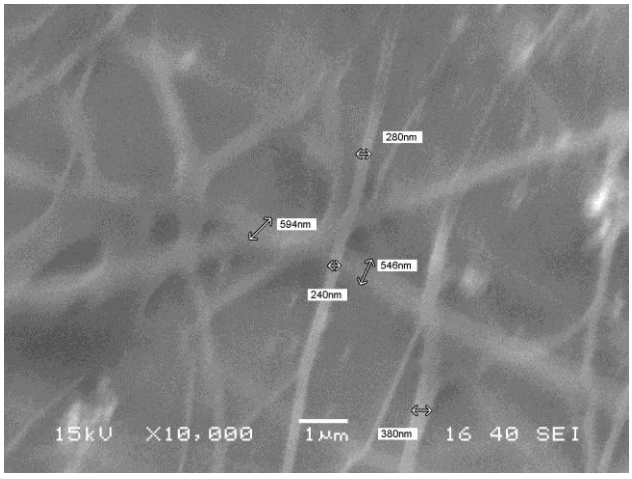


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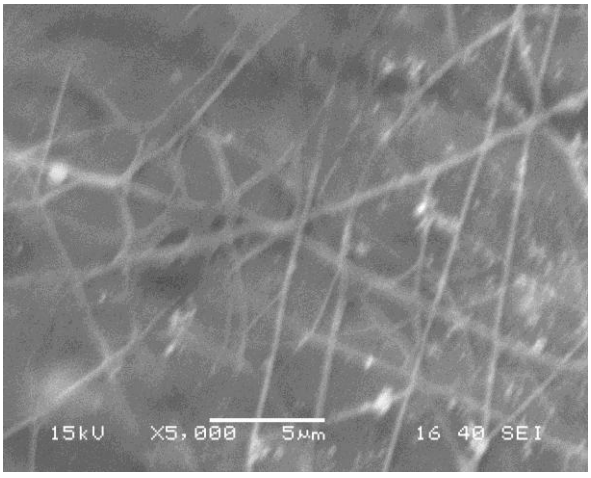
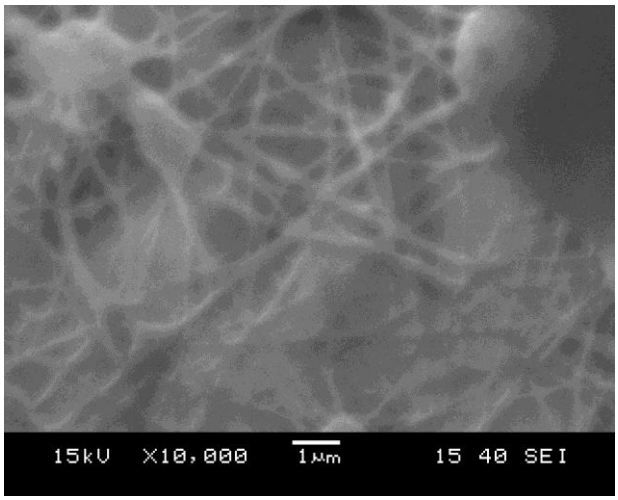
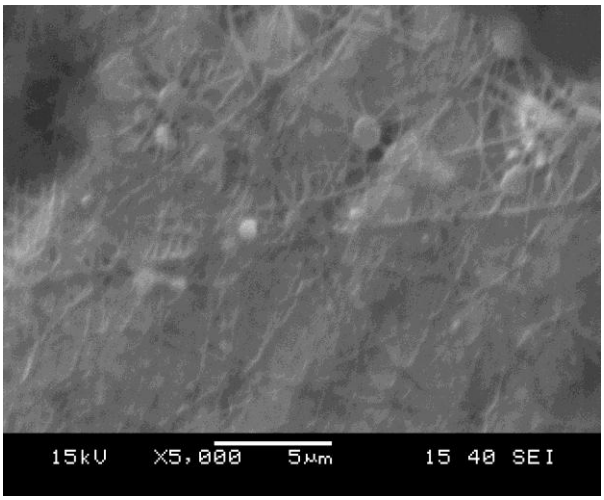



5 (h)

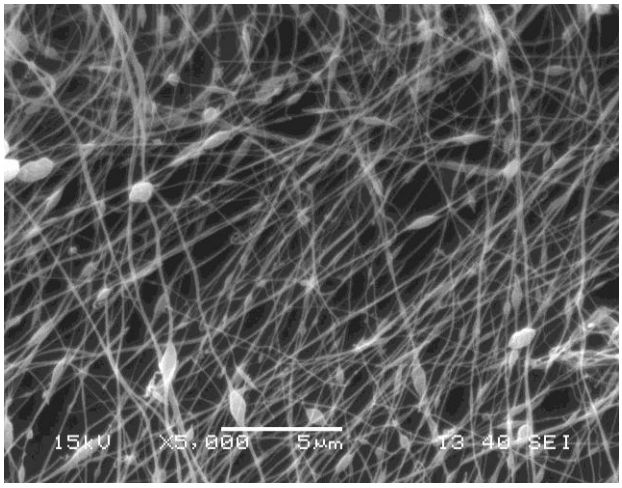



5 (i)

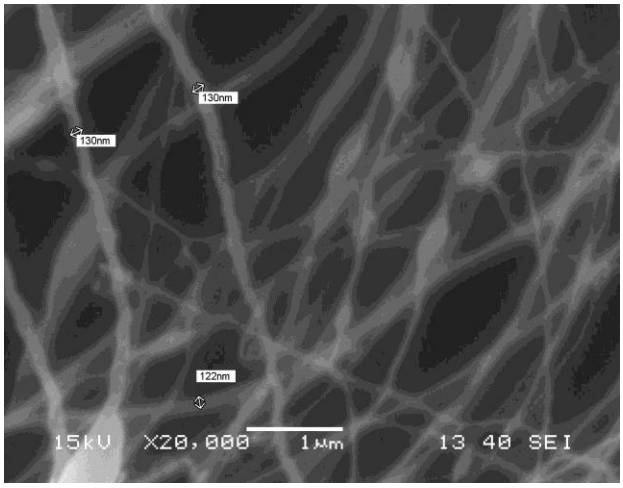



5 (j)

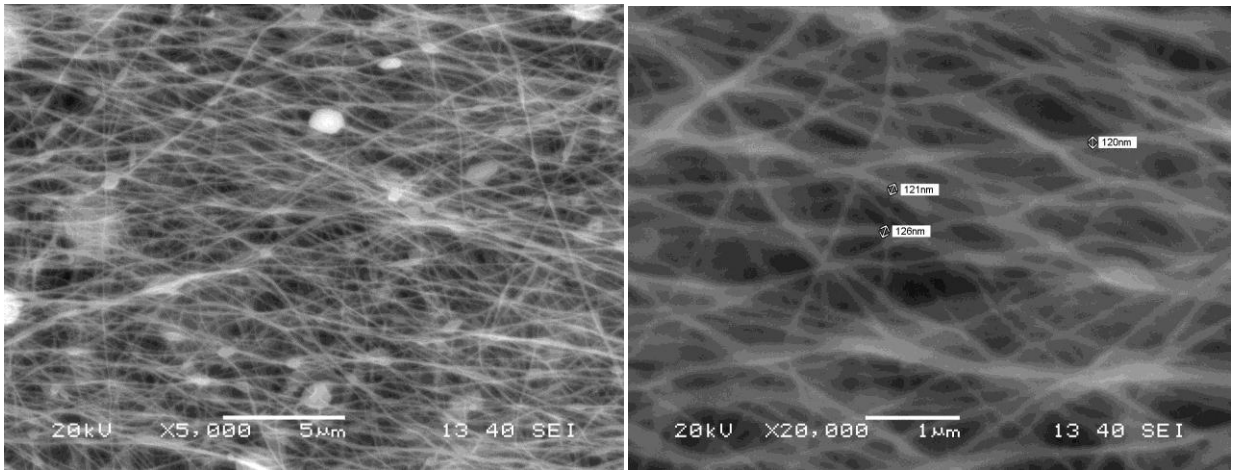
Figure. 5. SEM micrograph of electrospun CS: PVA fibres of ratio 10:90 (5a and 5b), 30:70 (5c and 5d), 35:65 (5e and 5f), 40:60 (5g and 5h) and 50:50 (5i and 5j)




6 (a)




6 (b)




6 (c) 6 (d)

Figure. 6. SEM micrograph of electrospun CS:SF:PEO fibres of ratio 1:1:1 (6a and 6b) and 2:2:1 (6c and 6d)

### *5.2.Phase Analysis*

X-ray diffractogram was obtained using X-ray diffractometer as shown in figure 7 and analysed for accessing the crystalinity of the sample. Phase change during blend formation and electrospinning process was studied by XRD.

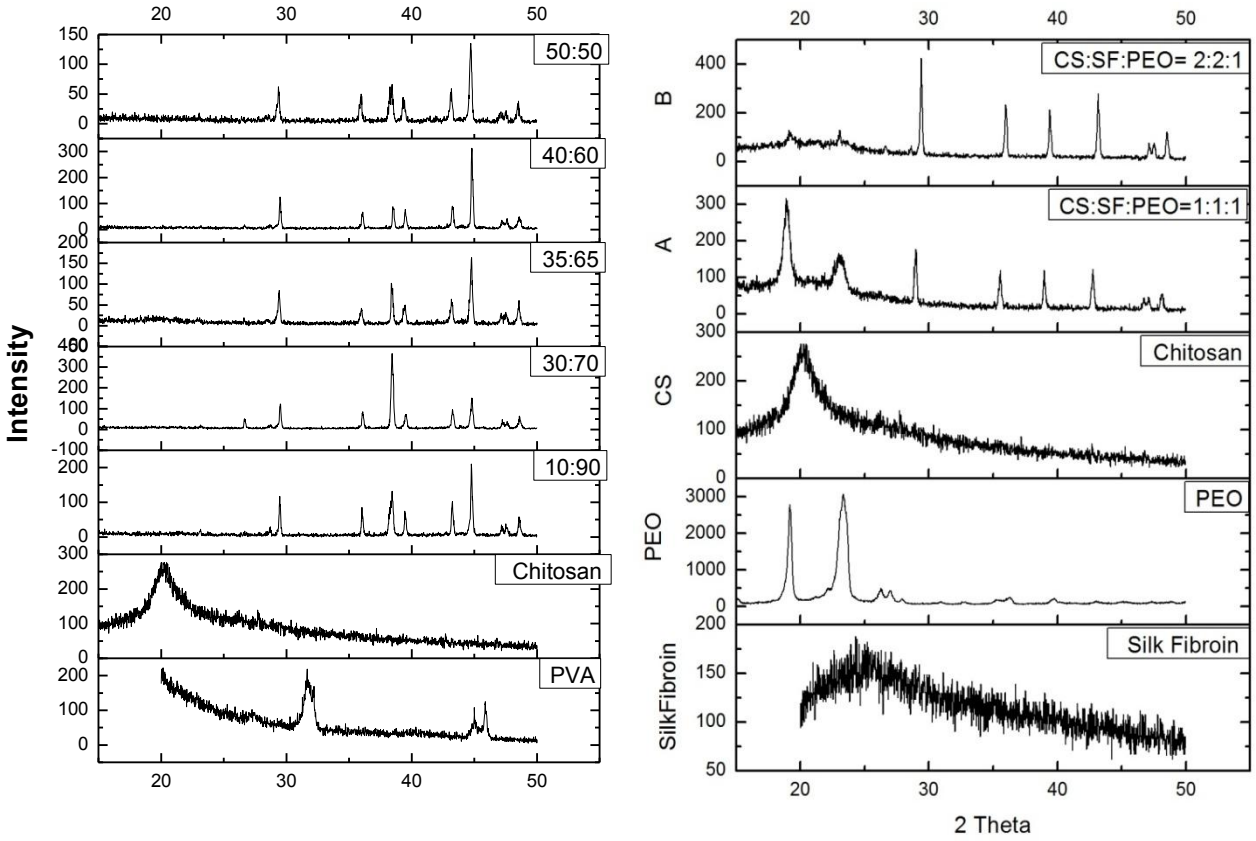
A. CS/PVA nanofibres- A dome shaped curve at $20^o$ in 35:65 (CS:PVA) sample depicted presence of chitosan in it, while peaks at $30^o$ and $48^o$ confirmed the presence of PVA in all the prepared composites (figure 7a).
B. CS/SF/PEO Nanofibres- Rise at $20^o$ confirmed the presence of chitosan in the sample and domes near $25^o$ showed the presence of silk fibroin in the blends. Both the blends showed all the major and minor peaks of PEO in the X-ray diffractogram (figure 7b).

Thus it was observed that the phase of the blends do not change after processing, and the diffractogram also confirmed the presence of all the components in the blends along with their crystalline nature when moulded into a scaffold by electrospinning. Crystallinity refers to the degree of structural order in a solid. In a crystal, the atoms or molecules are arranged in a regular, periodic manner. Polymer materials form crystalline regions, but generally long lengths of molecules usually prevent complete crystallization. Many polymers show semicrystalline behavior.

Standard Peak values for pure samples-

Table 3. Standard X-Ray Diffraction peak values for polymers used

| Component | 2 Theta value of | |
|---|---|---|
| | Major peak | Minor peak |
| **Chitosan (CS)** | $19^{o}$ | $9.4^{o}$ |
| **Polyvinyl alcohol (PVA)** | $30^{o}$ | $48^{o}$ |
| **Silk Fibroin (SF)** | $20.4^{o}$ | $24.5^{o}$ |
| **Polyethylene oxide (PEO)** | $30^{o}$ | $40^{o}$ |




7a 7 b

Figure 7. XRD analysis of electrospun CS:PVA blends (7a) and CS:SF:PEO blends (7b)

***FTIR analysis:***

The inter-molecular interaction can be determined by FTIR when two polymers are blended together for nanofibres fabrication [37]. In the case of a CS-PVA blends and CS, SF and PEO

composites used for electrospinning of nanofibers, the FT-IR analysis was based on the identification of absorption bands concerned with the vibrations of functional groups present in macromolecules. FT-IR spectra obtained from pure chitosan, Chitosan/PVA and pure PVA films is shown in Figure 8a and for CS/SF/PEO films is shown in Figure 8b.

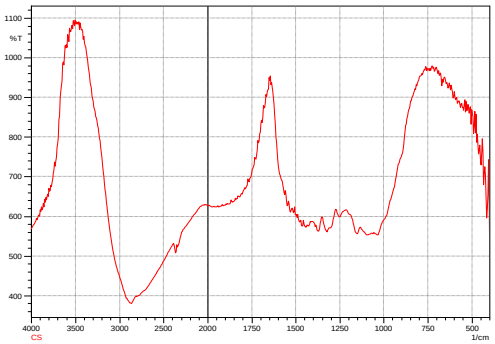


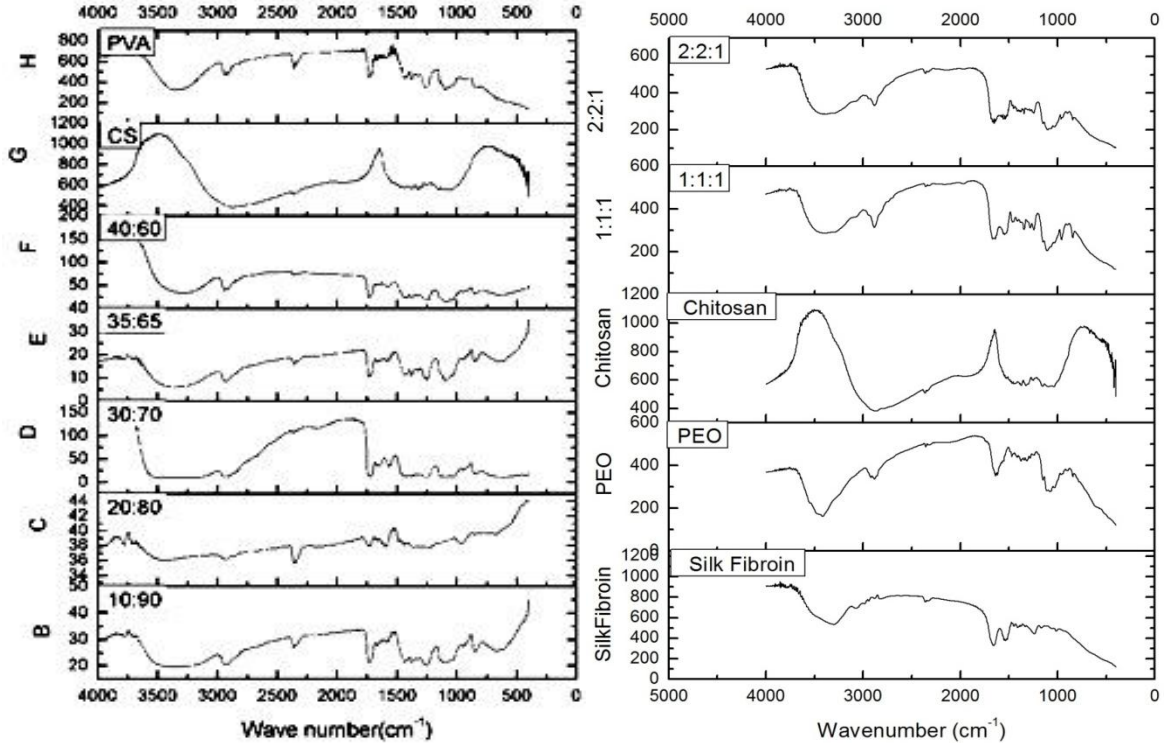
**Figure 9. FTIR spectrum of Chitosan**

For the spectrum of pure chitosan as seen in figure 9, the characteristic absorption bands of chitosan were observed at six locations. The vibrations of hydroxyl and free amine groups appeared at 3439 and 3300 $cm^{-1}$, respectively. The absorption bands at 1655, 1560 and 1381 $cm^{-1}$ indicated C=O stretching, –NH2 bending and C–O stretching of primary alcohol groups, respectively. The last one at 1152 $cm^{-1}$ represented -C-O-C- glycosidic linkage between chitosan monomers [40].

A. <u>CS/PVA blend-</u> In FTIR spectra of PVA all major peaks related to hydroxyl and acetate groups were observed. Large bands observed between 3550 and 3200 $cm^{-1}$ are linked to the stretching O–H from the intermolecular and intramolecular hydrogen bonds. The vibrational band observed between 2840 and 3000 $cm^{-1}$ refers to the stretching C–H from alkyl groups and the peaks between 1750–1735 $cm^{-1}$ are due to stretching C═O and C–O from acetate group remaining from PVA. The shift in the lower order of spectrum for the Chitosan/PVA blends is mainly due to primary alcohol and secondary alcohol interactions due to hydrogen bonding as earlier reported in studies of chitosan and PVA blends [41,42].

B. <u>CS/SF/PEO blend</u>- FTIR spectra of pure SF depicted four chatacteristic absorption bands for silk fibroin. At 700 $cm^{-1}$ amide-V group vibration while at 1260 $cm^{-1}$ was due to amide III vibration present in random coil of the structure. Amide II group present on beta-sheet conformation of SF showed absorption at 1525 $cm^{-1}$ position. While band at 1625 $cm^{-1}$ can be attributed to C═O bond vibration or if the molecule is in β-sheet conformation, then this band depicts amide-I bond vibration. For the spectrum of pure PEO, various band locations that signify particular functional group are, at 841 $cm^{-1}$ and

961 $cm^{-1}$ C-$H_2$, O-C-O bond stretching is depicted at 1101 $cm^{-1}$ and band at 2891 $cm^{-1}$ was linked to C-H bond stretching.




8 a 8b

Figure 8. FTIR spectra of CS/PVA (8a) and CS/SF/PEO (8b) blends

### *5.3.Swelling Ratio and Water Uptake Capacity*

A. CS/PVA blends- As shown in Figure 10, the swelling behaviour of CS/PVA scaffolds with different ratios with respect to time could be clearly distinguished. The blends containing CS less than 30% (w/w) showed good swelling. The other group of which the swelling ratios were as low as that of pure chitosan was the blends having chitosan composition more than 30%. This phenomenon can be attributed to the loss of gel-like structure after swelling.

   Water intake capacity of all the chitosan/PVA blends was approximately same and average value was 98.5576%.

B. CS/SF/PEO blends- weight of CS/SF/PEO blends were found to be lesser than 30% on swelling. Water uptake percent was also less as compared to CS/PVA blends.

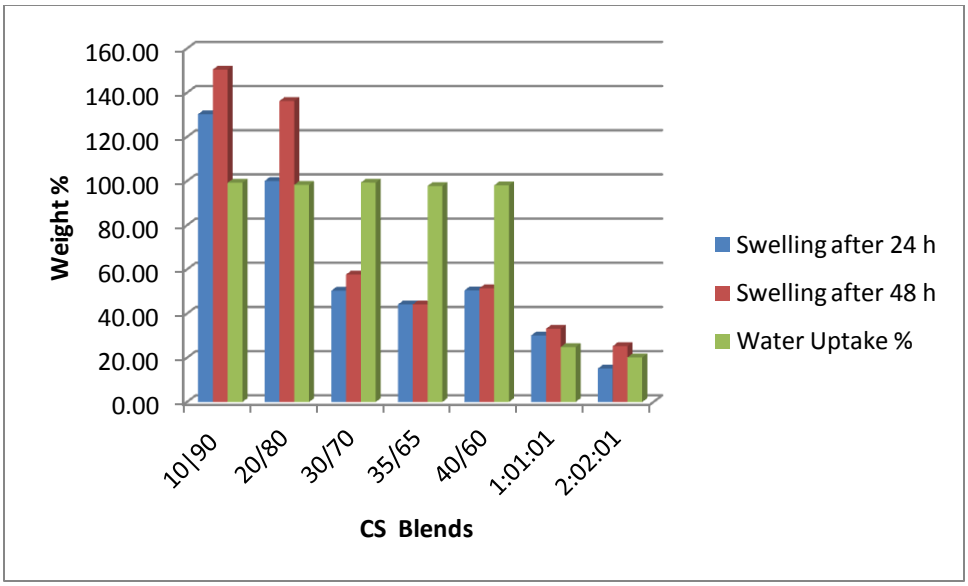



Figure 10. Swelling Ratio and Water Uptake Capacity of Chitosan composite scaffolds

### *5.4.Mechanical testing*

Due to very small dimension, the mechanical characterization of an individual nanofiber is a challenge for the existing test techniques. Figure 11 shows typical stress–strain curves of CS-composite nanofibers obtained by electrospinning for tissue engineering applications.

Following table 4 summarizes the tensile strengths obtained for different scaffolds composition at varying loads.

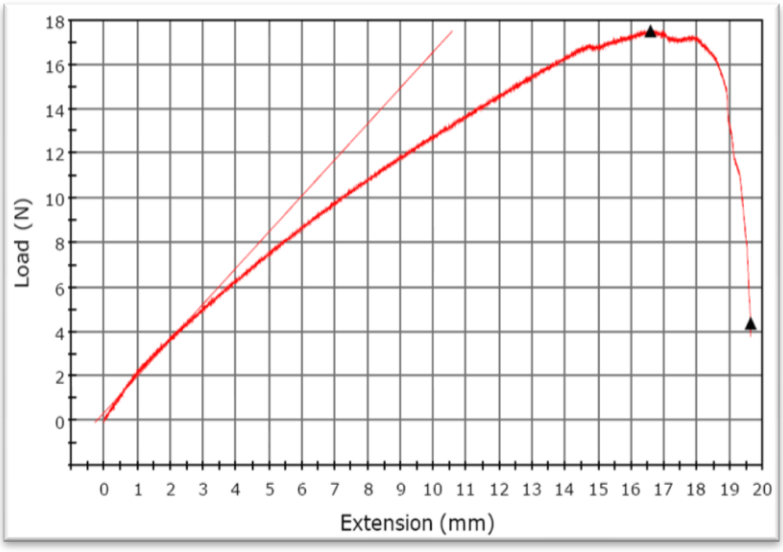



**Figure 11. Stress-strain curve of CS composite Nanofibers**

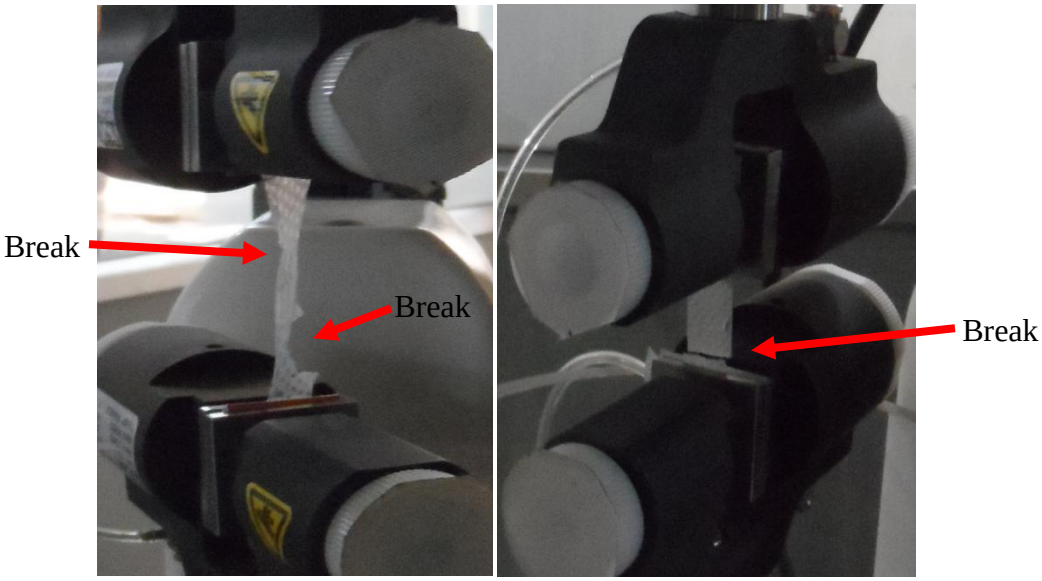
**Table 4. Tensile strength of composite scaffold at varying load**

| S. No. | Sample Label | Sample Thickness (mm) | Maximum Load (N) | Tensile Strength (MPa) | Load at Break (N) | Tensile strain at break (%) | Modulus (kPa) |
|---|---|---|---|---|---|---|---|
| | **CS:PVA** | | | | | | |
| 1. | 10:90 | 0.10 | 5.79 | 3.22 | 1.44 | 38.91 | 23408.32 |
| 2. | 20:80 | 0.30 | 13.98 | 4.66 | 4.77 | 109.52 | 7572.45 |
| 3. | 30:70 | 0.25 | 5.93 | 5.93 | 5.84 | 25.67 | 37687.91 |
| 4. | 35:65 | 0.31 | 18.44 | 6.15 | 10.49 | 83.44 | 12385.66 |
| 5. | 40:60 | 0.02 | -- | -- | -- | -- | -- |
| | **CS:SF:PEO** | | | | | | |
| 1. | 1:1:1 | 0.055 | 2.89 | 5.79 | 2.77 | 1.36 | 425990.53 |
| 2. | 2:2:1 | 0.02 | -- | -- | -- | -- | -- |

It was observed that the tensile strength of the nanofibres largely depend upon their geometry and composition.

A. CS/PVA blends- A trend of increase in tensile strength was observed with increase in CS composition in the blends. The break at stretch seen in each case followed the trend of non uniform sheets. In case of 40:60 nanofiber sheet, lesser thickness value (0.02mm) limited its tensile testing by this method.
B. CS/SF/PEO blends- Nanofiber sheets comparising the components in 1:1:1 ratio, showed less tensile strength. And 2:2:1 composition nanofiber sheet was very thin and thus its tensile testing could not be done following this method. It can be concluded that the presence of SF in the composite makes the fibres comparatively brittle, and thus decreases the tensile strength.

Non-uniform break on stretching can be explained by the relation that samples comparises of unaligned nanofibres, as revealed by SEM micrographs, thus force gets distributed in different directions during stretching, as compared to the aligned fibres, where the distribution of force is in a particular direction resulting into straight cut, as shown in the figure 12.




**12 a** **12 b**

**Figure 12. Stretching and break of un-aligned (12a) and aligned (12b) fiber sheets**

The established methods and standards for determining the mechanical behaviour of conventional fibers are inadequate in the case of manipulation or testing of nanofibres. It has been found that tensile strength of nanofibrous mat was less than that of a cartilage, which should be near 40 MPa. This experiment was performed on non-aligned fibers, since fiber orientation plays a major role in determining tensile strength of any material. Fibers show good tensile strength when pulled along the direction of fibers. Thus an attempt to form well aligned nanofibres would compliment in achieving the desired mechanical strength.

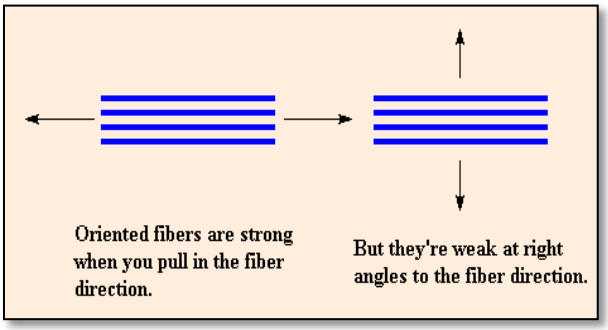



**Figure 13. Relation between fiber orientation and tensile strength**

### *5.5.Biodegradation*

The biodegradation results are shown in Figure 14 and 15.

A. CS/PVA blends- PVA scaffolds incubated in SBF had the highest weight reduction and were completely degraded after 30 mins. However, the addition of chitosan reduced the degradation of scaffolds in SBF solution. Regarding the stability of scaffolds which was higher than that of pure PVA scaffolds, the results obtained, proved to be the vital characteristic since it is well-known that the degradation rate of PVA scaffolds was very fast, hence the addition of chitosan could prolong the biodegradability of scaffolds. Most of the scaffolds showed complete degradation within 30 days of observation. While blend ratio 30:70 and 35:65 (CS: PVA) were disintegrated into soft smaller pieces whose weighing became difficult beyond 30 days.

B. CS/SF/PEO blends- Scaffolds were incubated in SBF and it was observed that degradation started immediately on the first hour and then there was no noticeable change till 24hr. Their degradation resumed after that, and following continuous weight loss the scaffolds were completely degraded on 6$^{th}$ day of the start of degradation study.

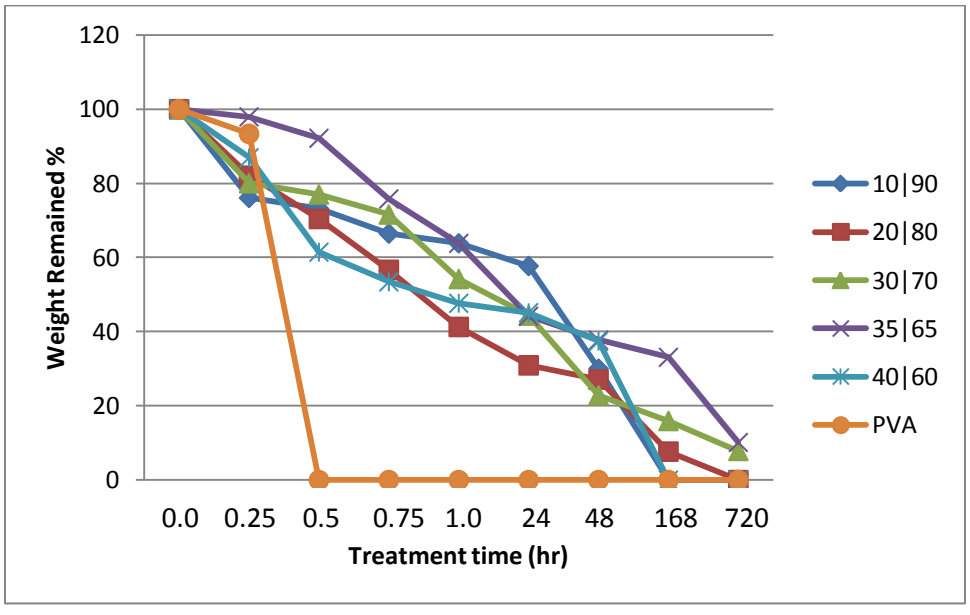



**Figure 14. In vitro biodegradation of CS/PVA scaffolds with different blending compositions**

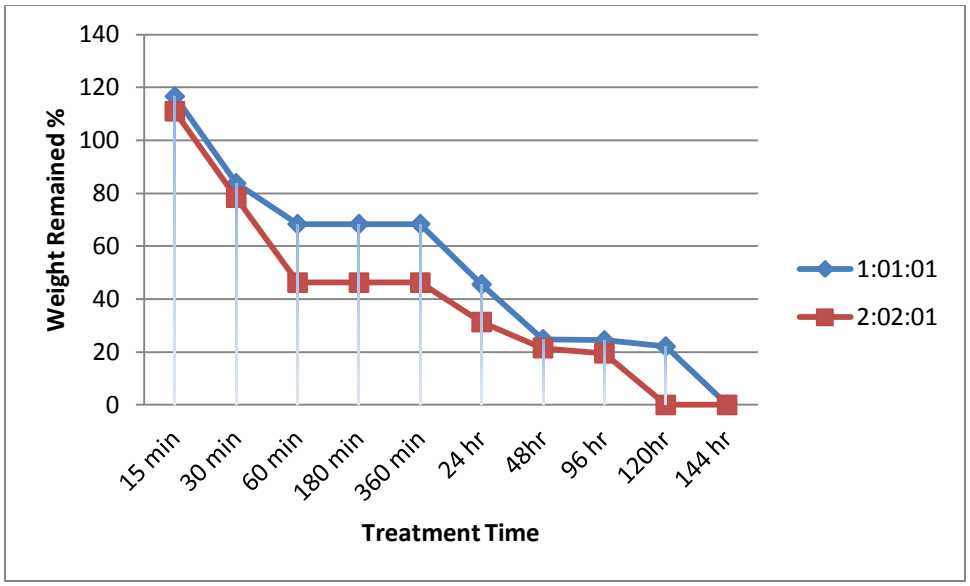



**Figure 15. In vitro biodegradation of CS/SF/PEO scaffolds with different blending compositions**

## 6. *in-vitro* biocompatibility study

Cells did not attach to the scaffold surface. Cell growth was not observed in the prepared scaffolds. The media might be lacking adequate growth factors required for cell attachment, growth and proliferation. The scaffold might have not been neutralized, since the use of acetic acid as solvent has made the formulation more acidic and thus unsupportive for cellular growth.

Table 5. Combined Results for various characterization techniques

| Characterization Technique | Property of comparison | CS/PVA blends | CS/SF/PEO blends | Remarks |
|---|---|---|---|---|
| **Rheology behavior** | Viscosity Measurement | 2 Pa sec – 8.261 Pa sec | 1.075 Pa sec – 5.72 Pa sec | Increasing with increasing ratio of PVA |
| **Morphology Analysis (SEM)** | Fibre Diameter (Average) | 300 nm | 120 nm | Addition of SF results into finer nanofibers |
| **X-ray Diffraction (XRD)** | Diffraction pattern and phase change | No phase change | No phase change | Composites contain the components blended prior to electrospinning |
| **FTIR** | Functional group detection | Present | Present | Composites contain functional groups of their pure form components |
| **Swelling Ratio** | Swelling in weight % | Good swelling observed (~ 88.03%) | Lesser swelling (~29.202%) | Best result for CS/PVA 10:90 and 30:70 |
| **Water Uptake capacity** | Water uptake Percent (Hydrophilicity) | (98.5576%) | Lesser uptake (22.53%) | CS/SF/PEO blends are less hydrophilic than CS/PVA blends |
| **Mechanical Testing** | Tensile strength | 3.22- 6.15 MPa Average 4.99 MPa | 5.79 MPa | Nanofibres formed by electrospinning possess considerable load bearing capacity |
| **Biodegradation** | Weight loss in SBF wrt time | Complete Degradation after 30 days | Complete degradation in 6 days | Weight loss rate of CS/PVA scaffold is lower than that of CS/SF/PEO |

**CHAPTER- 5**

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# *Conclusion*

In this study, chitosan was blended with PVA and SF polymers. The polymer blends were successfully electrospun to fabricate CS/PVA and CS/SF/PEO nanofibrous scaffolds. The CS/SF/PEO scaffolds have been found to exhibit better physicochemical properties, compared with CS and CS/PVA scaffolds, in order to meet the requirements of bone and cartilage tissue regeneration. Efforts to improve mechanical properties of CS based composites are essential for its application in bone tissue engineering. Cell study has confirmed that these blends possess appreciable biological properties like biocompatibility and biodegradability. The spreading of cells on the scaffold surface was a bit non uniform as observed by preliminary cell culture study for which and detail study is required to specify their use for specific cell types.

# Future Prospects

Chitosan can be blended with various polymers as performed in present study; its blending with ceramics can be studied to fabricate a stronger material which could be used in tissue engineering for specific tissues or cell types. Detail in vitro cell growth is required to ascertain the scaffold for particular tissue regeneration.

# *Publication*

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P. Agrawal, K. Pramanik, **Preparation and Characterization of Electrospun Chitosan- Poly Vinyl Alcohol Nanofibres by Nozzle Free Electrospinning Method,** *Journal of Fibers and Polymers.*

**-Communicated**

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